# PATENT COOPERATION TREATY

# PCT



## INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

(Chapter II of the Patent Cooperation Treaty)

(PCT Article 36 and Rule 70)

| Applicant's or agent's file reference<br>10355.204-WO | **FOR FURTHER ACTION** | See Form PCT/IPEA/416 |
|---|---|---|
| International application No.<br>PCT/DK2004/000699 | International filing date *(day/month/year)*<br>13.10.2004 | Priority date *(day/month/year)*<br>16.10.2003 |
| International Patent Classification (IPC) or national classification and IPC<br>C12N15/10, C12N15/75, C12Q1/68 | | |
| Applicant<br>NOVOZYMES A/S et al. | | |

1. This report is the international preliminary examination report, established by this International Preliminary Examining Authority under Article 35 and transmitted to the applicant according to Article 36.
2. This REPORT consists of a total of 6 sheets, including this cover sheet.
3. This report is also accompanied by ANNEXES, comprising:
   a. ☒ *sent to the applicant and to the International Bureau)* a total of 3 sheets, as follows:
      ☒ sheets of the description, claims and/or drawings which have been amended and are the basis of this report and/or sheets containing rectifications authorized by this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions).
      ☐ sheets which supersede earlier sheets, but which this Authority considers contain an amendment that goes beyond the disclosure in the international application as filed, as indicated in item 4 of Box No. I and the Supplemental Box.
   b. ☐ *(sent to the International Bureau only)* a total of (indicate type and number of electronic carrier(s)) , containing a sequence listing and/or tables related thereto, in computer readable form only, as indicated in the Supplemental Box Relating to Sequence Listing (see Section 802 of the Administrative Instructions).

4. This report contains indications relating to the following items:

| | | |
|---|---|---|
| ☒ | Box No. I | Basis of the opinion |
| ☐ | Box No. II | Priority |
| ☐ | Box No. III | Non-establishment of opinion with regard to novelty, inventive step and industrial applicability |
| ☐ | Box No. IV | Lack of unity of invention |
| ☒ | Box No. V | Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement |
| ☐ | Box No. VI | Certain documents cited |
| ☐ | Box No. VII | Certain defects in the international application |
| ☐ | Box No. VIII | Certain observations on the international application |

| Date of submission of the demand | Date of completion of this report |
|---|---|
| 16.08.2005 | 20.01.2006 |
| Name and mailing address of the international preliminary examining authority:<br>European Patent Office - P.B. 5818 Patentlaan 2<br>NL-2280 HV Rijswijk - Pays Bas<br>Tel. +31 70 340 - 2040 Tx: 31 651 epo nl<br>Fax: +31 70 340 - 3016 | Authorized Officer<br>Aslund, J<br>Telephone No. +31 70 340-4393 |

## Box No. I Basis of the report

1. With regard to the **language**, this report is based on the international application in the language in which it was filed, unless otherwise indicated under this item.

   ☐ This report is based on translations from the original language into the following language , which is the language of a translation furnished for the purposes of:
   - ☐ international search (under Rules 12.3 and 23.1(b))
   - ☐ publication of the international application (under Rule 12.4)
   - ☐ international preliminary examination (under Rules 55.2 and/or 55.3)

2. With regard to the **elements*** of the international application, this report is based on *(replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report)*:

   **Description, Pages**

   1-36 as originally filed

   **Claims, Numbers**

   1-22 received on 19.08.2005 with letter of 16.08.2005

   ☒ a sequence listing and/or any related table(s) - see Supplemental Box Relating to Sequence Listing

3. ☒ The amendments have resulted in the cancellation of:
   - ☐ the description, pages
   - ☒ the claims, Nos. 23-25
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:
   - ☐ any table(s) related to sequence listing *(specify)*:

4. ☐ This report has been established as if (some of) the amendments annexed to this report and listed below had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).
   - ☐ the description, pages
   - ☐ the claims, Nos.
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:
   - ☐ any table(s) related to sequence listing *(specify)*:

* *If item 4 applies, some or all of these sheets may be marked "superseded."*

**Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

1. Statement

| | | | |
|---|---|---|---|
| Novelty (N) | Yes: | Claims | 7-15, 18-20 |
| | No: | Claims | 1-6, 16, 17, 21-22 |
| Inventive step (IS) | Yes: | Claims | |
| | No: | Claims | 1-22 |
| Industrial applicability (IA) | Yes: | Claims | 1-22 |
| | No: | Claims | |

2. Citations and explanations (Rule 70.7):

**see separate sheet**

## Supplemental Box relating to Sequence Listing

**Continuation of Box I, item 2:**

1. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application and necessary to the claimed invention, this report has been established on the basis of:

   a. type of material:

   ☒ a sequence listing

   ☐ table(s) related to the sequence listing

   b. format of material:

   ☒ in written format

   ☒ in computer readable form

   c. time of filing/furnishing:

   ☒ contained in the international application as filed

   ☒ filed together with the international application in computer readable form

   ☐ furnished subsequently to this Authority for the purposes of search and/or examination

   ☐ received by this Authority as an amendment on

2. ☐ In addition, in the case that more than one version or copy of a sequence listing and/or table(s) relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional observations, if necessary:

**Citations**

The following documents (D) are cited:

D1:NOONE DAVID ET AL: "YkdA and YvtA, HtrA-like serine proteases in Bacillus subtilis, engage in negative autoregulation and reciprocal cross-regulation of ykdA and yvtA gene expression" JOURNAL OF BACTERIOLOGY, vol. 183, no. 2, January 2001 (2001-01), pages 654-663, XP002266733 ISSN: 0021-9193

D2:GRIFFITH DOUGLAS ANDREW ET AL: "A novel yeast expression system for the overproduction of quality-controlled membrane proteins." FASEB JOURNAL, vol. 17, no. 4-5, March 2003 (2003-03), page Abstract No. 369.8, XP008026411 FASEB Meeting on Experimental Biology: Translating the Genome;San Diego, CA, USA; April 11-15, 2003 ISSN: 0892-6638 (ISSN print)

D3: GRIFFITH D A; DELIPALA C; LEADSHAM J; JARVIS S M; OESTERHELT D: A novel yeast expression system for the overproduction of quality-controlled membrane proteins FEBS LETTERS 2003-10-09, VOL 553 (1-2) pages 45-50

D4: Lesley SA, Graziano J, Cho CY, Knuth MW, Klock HE: Gene expression response to misfolded protein as a screen for soluble recombinant protein.
Protein Eng 2002, 15:153-160

D5: Waldo GS: Genetic screens and directed evolution for protein solubility.
Curr Opin Chem Biol 2003 (available online January 7), 7:33-38

D6: Jones et al (1997) "The chaperone-assisted membrane release and folding pathway is sensed by two signal transduction systems", Embo J 16, 6394-6406

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**Novelty**

Claim 1 has been amended to specify screening of a gene library by testing the activity of a secretion stress promoter. However, D2 discloses host cells where the activity of the unfolded protein response (UPR) is monitored using a UPR element-LacZ fusion. This response is considered equivalent to "secretion stress". D2 discloses "different copy numbers of an integrating TeP2 vector". Different copy numbers of the same gene can be

considered a gene library. A disclosure similar to D2, but more detailed, can be found in D3 page 47 column 2.
Thus, amended claim 1 lacks novelty over D2, D3.
D6 (pages 6399, Fig 5) discloses activation of a degP-lacZ reporter upon overproduction of either PapE or PapG. Page 6399 column 2 last paragraph, further states that "The presence of misfolded or partially denatures proteins in the periplasm is thought to be a signal that leads to activation of degP transcription". In fact, degP is also known as htrA - i.e. one of the genes, the promoter of which is proposed to be used for monitoring of secretion stress in the present application. In any case, D6 discloses expression of several genes (i.e. a library) in a host where the activity of a promoter responsive to secretion stress can be monitored. Thus, also D6 is novelty destroying for claim 1.

**Inventive step**

Claims 7-15, 18-20 specify features such as the sequence of the promoter. These claims lack an inventive step for the following reasons:
The overall strategy of monitoring a host cell's response to protein overproduction by testing the activity of stress promoters has been reviewed in D4, D5. Many stress promoters (e.g. promoters of the heat shock response) respond to the presence of unfolded proteins in a cell - this includes unfolded proteins generated using overexpression strategies.
In order to monitor such stress induced by unfolded proteins, a reporter gene is typically expressed under the control of e.g. a heat shock promoter. Clones (or growth conditions) that allow expression of a target protein without triggering the stress response can then be selected.
The present application differs from D4, D5 in that secreted proteins are concerned. In view of the fact that D1 discloses the same secretion stress promoter as in the present application, it would be a trivial task for a person skilled in the art to adapt the approaches from D4 (or D5) to establish a method for screening recombinant bacterial host cells by testing the activity of a secretion stress promoter. Hence, an inventive step is denied.